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MICROSCOPY.[1]

OSMIC ACID AND MERKEL'S FLUID AS A MEANS OF DEVELOPING NASCENT HISTOLOGICAL DISTINCTIONS.[2]—In preparing embryological material for the microtome and the microscope, our choice of preservative fluids depends on the advantages offered in three principal directions. We inquire first of all what reagent, or combination of reagents, will best preserve the natural *form, relations* and *internal structure.* We next endeavor to ascertain which of the fluids appearing to satisfy the first point will leave the preparation in the most favorable condition for sectioning; and, finally, we have to consider the *differentiating capacity* of the fluids, and the conditions under which the highest differential effects can be obtained. This highly important quality, which belongs, in varying degree, to all hardening and staining reagents, serves two general purposes, one of which is purely histological, the other strictly embryological. In the one case, the aim is to sharpen the definition of individual elements, and to strengthen histological distinctions; in the other, the object is to demonstrate those subtle and imperceptible differences in the constitution of embryonic cells, which furnish the earliest premonitions of their histological destiny. The histologist deals with the first class of distinctions—the embryologist must deal with both. The embryologist cannot stop with the study of structure and topographical relations, as they exist in any particular stage; he is compelled to follow the entire developmental history of the cells, from their most indifferent up to their most highly specialized condition. Beginning with material more or less homogeneous in aspect, he finds it necessary to forestall development, and seeks to bring out distinctions that have not yet ripened into morphological definition. In short, his task is no less than that of discovering, by chemical means, promorphological conditions, which shall reveal the destination of cells before nature has given them any definite histological stamp. The means that suffice to demonstrate fully formed tissue elements are not always identical with those required in tracing their histogenetic development. As yet we know very little about the capacity of different preservative fluids in the very important work of developing nascent histological distinctions. It is often at the expense of much time and patience that reagents are found which combine the first two qualifications we have mentioned, and the experimenter who has been so far successful too frequently flatters himself that he has reached the highest rung in the ladder of technical bliss, if his preparations admit of being "sliced like cheese or cartilage." But one requires no very large amount of knowledge of the aims, and experience in the ways and means, of embryological research, in order to understand that the investigator's art does not culminate in sections of cheese-like homogeneity. To be able, through serial sections, to lay bare each individual cell of a complicated organism is certainly a great triumph in microtomy; but such a feat may be, as it not infrequently has been, accomplished without leading to any important results, and simply because the methods of preparation have not been selected with a view to secure the needed differential effects.

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[1] Edited by Dr. C. O. WHITMAN, Mus. Comp. Zool., Cambridge, Mass.

[2] Read before the American Society of Naturalists, December 30, 1885.

Having defined a special aim in the use of embryological methods, it remains only to consider the practical side of the subject. The differential effects of most preservative fluids, when used singly, are extremely weak, and often quite inappreciable. To be of service, they must be strengthened or reinforced by some happy combination of reagents, discoverable only by experiment. Differential results are generally sought for through metallic impregnations and through various methods of staining, as double staining, multiple staining, overstaining followed by partial decoloration, etc. But I am not aware that such means alone are sufficient for the special purpose under consideration. In order to demonstrate differences, not of form, but of molecular constitution, the foundation for the desired effects must be laid in the process of hardening. Staining reagents may then serve to complete the work.

As an example of what may be accomplished in this way, I will give briefly my own experience with osmic acid and the so-called Merkel's fluid, which is a mixture in equal parts of chromic acid (¼ p. c) and of platinum chloride (¼ p. c.). I have tested these reagents with three different classes of eggs, and have obtained important results, some of which have already been published. In the case of pelagic fish-eggs, with which my first experiments were made, the method of procedure is as follows: The eggs, with a little sea-water, are placed in a watch-glass; then, by the aid of a pipette, a quantity of osmic acid ($\frac{1}{2}$ p. c.) equal (as nearly as one can judge) to that of the sea-water is added. At the end of from five to ten minutes, the eggs are washed quickly in clean water, and transferred to a chrome-platinum solution, differing from Merkel's mixture only in having a higher per cent of chromic[1] acid, where they may remain from one to three days. After this treatment, the blastoderm may be easily freed from the yolk, and, after a thorough washing in clear water for a number of hours, the preparation may be passed through the usual grades of alcohol, stained and sectioned, or mounted in toto. The osmic acid fixes the natural form and structural features of the egg perfectly, and the mixture of chromic acid and platinum chloride completes the work of hardening, and at the same time removes much of the brown or black color imparted by the first reagent. I have tried various other reagents after the osmic acid, but with far less satisfactory results. Picro-sulphuric acid, instead of arresting the blackening process of the osmic acid, increases it. Simple chromic acid arrests the blackening, but does not remove it (as does Merkel's fluid), and causes considerable contraction. Müller's fluid, recommended by Henneguy, is equally unsatisfactory. By this method a very marked differentiation is generally obtained as early as the sixteen-cell stage, the four central cells showing a very light brown shade, while the twelve peripheral cells have a much deeper shade. In later stages of cleavage, the distinction between central and marginal cells becomes still stronger, so that it becomes possible to trace the entire history of the origin of the so-called parablast, over which there have been so many controversies. The very difficult question as to the precise origin of the permanent entoderm is not settled by this method.

The same reagents may be successfully applied to the eggs of Clepsine; but here the mode of procedure is somewhat different, as regards Merkel's fluid. This mixture, employed at its normal strength, is allowed to work from one to two hours only. The differential effects are here very marked, extending not only to the different germ-layers, but even to cell-groups destined to form the central nervous system, the nephridial organs, larval glands, etc. None of the methods hitherto employed with these eggs has given results at all comparable with those I have mentioned.

In the case of the frog's eggs, I allow the osmic acid from twenty to twenty-five minutes, then transfer directly to the chrome-platinum solution employed with fish-eggs (twenty-four hours). The eggs are next placed in water and freed from their gelatinous envelopes by the aid of sharp needles and a dissecting microscope. After washing in flowing water for at least two hours, the eggs may be treated with alcohol and stained according to desire. My experiments with these eggs have not yet been carried very far, and I can only say that the material, so far as examined, has turned out well. If the sectioning is not delayed too long, no disagreeable effects of crumbling will be experienced. —*C. O. Whitman.*

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[1] A one per cent solution is used in place of the normal $\frac{1}{4}$ p. c. solution.

THE FUNCTION OF THE COMPOUND EYE.—It is held by Exner, Carrière, and others that the compound eye does not distinguish the *forms*, but only the *movements* of objects. The eye would thus be merely an organ of orientation, capable of recognizing differences in the intensity of light. Plateau[1] has undertaken a series of interesting experiments designed to test the validity of this view. The method of experimentation was as follows:

A room five meters square is furnished with two windows, which face the west. The windows are provided with shutters, by means of which the room can be made dark. In each shutter a hole is cut large enough to receive a pane of ground glass. The vertical distance from the floor to the center of each glass is $1.75^{m,}$ and the horizontal distance between the centers of the two panes is $2.30^{m.}$ The amount of light admitted is regulated by means of black pasteboard diaphragms, which are fitted to slide in front of the glass. The diaphragm covering the left pane is perforated with a single hole, which is amply large to allow the insect to pass through in full flight. The size of the opening is varied by using different diaphragms. The diaphragm covering the right pane is perforated with a number of small holes, through which the insect could not pass. This diaphragm remains the same through all the experiments.

To begin with, the single opening in the left diaphragm is made 10^{cm} square, and the right diaphragm is perforated with 100 small holes, each 1^{cm} square, and separated by spaces 1^{cm} in width. The 100 holes thus represent the same surface as the large opening, but the amount of light that passes the former is considerably less than that which passes the latter. In successive experiments with different diurnal insects (Diptera, Hymenoptera, Lepidoptera, Coleoptera, &c.), the size of the hole in the left diaphragm is varied, so that the amount of light is sometimes greater, sometimes less than that of the right diaphragm.

If, under these conditions, an insect let loose at the side of the room opposite the windows, invariably flies to the large opening, then we might conclude, according to Plateau, that it distinguishes the forms of objects; but if it often makes the mistake of flying against the surface perforated with holes too small to give it passage, we may conclude that it does not distinguish form, but is guided by the intensity of the light. The experiments show that the flight is directed, in the majority of cases, towards the more intense light, and hence Plateau concludes that the view before stated in regard to the function of the compound eye is correct. He further announces his conviction that the simple eyes are rudimentary organs that serve no important purpose. This view rests on the fact that if the ocelli are covered with opaque black varnish, the insect guides its course in the same manner as before.

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[1] Bull. de l'Acad. roy. de Belg., 3^{me} sér. t. x, No. 8, 1885.

While these experiments may be said to favor the conclusion arrived at by Plateau, they do not, in my opinion, furnish decisive evidence. It would be quite within the range of possibilities, that the insect distinguished perfectly well the *forms* of both the large and small holes, without taking in the relation of its own size to that of the hole through which it sought to escape. The power to distinguish forms is not tantamount to a knowledge of relations that could only be learned by experience and reflection.

A METHOD OF BLEACHING WINGS OF LEPIDOPTERA TO FACILITATE THE STUDY OF THEIR VENATION.[1]—In the common method of destroying the scales on the wings of Lepidoptera, for the purpose of studying their venation, by means of caustic alkaline solutions, there is danger of not arresting the action at the proper moment, and consequently of destroying not only the portions which it is desirable to remove, but also the scale-supporting membrane, and even the delicate veins themselves. An application of a modification of the chlorine bleaching process, commonly used in cotton bleacheries, obviates the necessity of removing the scales, and leaves the wing perfect.

The most convenient method of applying the chlorine is as follows: The wings must first be soaked a few moments in pure alcohol in order to dissolve out the oily matter in them. If this is not done the surface of the wings acts as a repellent, and will not be moistened by an aqueous solution. When the wings have become thoroughly soaked by the alcohol they are ready to be removed to a solution of common bleaching powder. This bleaching powder is sold by druggists as "chloride of lime," but it is really a mixture of calcic hypochlorite, calcic chloride, and calcic hydrate. Ten parts of water dissolve the first two compounds, leaving nearly all the third suspended in the solution. The solution should be made with cold water, filtered, and kept in a tightly corked bottle until required for use. When the wings are transferred to this solution the bleaching commences, and in an hour or two the wings are devoid of markings, although the veins retain a light brown color. This is due to the fact that chlorine cannot quite decolorize animal matter, or any substance containing nitrogen, as it does vegetable tissue.

After the color has sufficiently disappeared from the wings they should be transferred to a wash composed of one part of strong hydrochloric acid to ten parts of water. And here it may be added that in case the bleaching does not readily commence upon immersion in the bleaching solution, the action may be hastened by a previous dipping in the dilute hydrochloric acid. In the bleaching solution a crust of calcic carbonate, formed by the union of the calcic hydrate of the solution and the carbonic dioxide of the air, is deposited on the wings, and this calcic carbonate the final wash in dilute acid will remove. As soon as the calcic carbonate has disappeared, and all bubbling, consequent upon its decomposition by the hydrochloric acid, has ceased, the wings should be well soaked in pure water. They may then be secured on cards with a mucilage of gum tragacanth; or upon glass by the proper transfers, through alcohol and chloroform, to Canada balsam.

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[1] G. Dimmock, Proceedings of the American Association for the Advancement of Science, Detroit meeting, August, 1875.

A solution of sodic hypochlorite, known as *Eau de Labarraque* or a solution of potassic hypochlorite, known as *Eau de Javelle*, when used in place of the solution of bleaching powder does not leave a deposit of calcic carbonate on the wings and thus dispense with the wash of dilute acid. A solution of zinc hypochlorite acts more delicately than a solution of sodic hypochlorite, and may be used in place of the latter, as may also solutions of aluminic hypochlorite, or magnesic hypochlorite.

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SCIENTIFIC NEWS.

— The recent meeting of the Society of Naturalists, held at Boston, December 29 and 30, will long be remembered with pleasure by those who had the good fortune to be present. The excellent plan of the founders of the society of limiting the range of the papers to the discussion of methods of teaching and research, leaves but small foothold for bores, and, indeed, the series of papers furnished an exhilarating succession of suggestive and easily grasped ideas. Most of the sessions were held in the physiological lecture-room of the new Harvard Medical School building and just adjoining the laboratory of Dr. Bowditch, which is probably unparalleled for its wealth of ingenious and effective apparatus, designed and made on the spot. The courtesies of the Harvard members of the society very agreeably occupied the hours not strictly devoted to business.

— Professor T. J. Burrill deals, in the *Botanical Gazette*, p. 334, with two mechanical effects of cold upon trees—the radial splitting of wood and bark, and the separation of bark or wood layers in a concentric way.

The first is explained by water freezing in plates parallel to the surface of an organ, and then, additions being made to the base,